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Hillslope Dissolved Organic Matter Transport and Transformation in a Semi-Arid Headwater Catchment

Margaret Anne Burns
University of Colorado Boulder, margaret.a.burns1@gmail.com

HILLSLOPE DISSOLVED ORGANIC MATTER TRANSPORT AND TRANSFORMATION IN A SEMI-ARID HEADWATER CATCHMENT

by

MARGARET ANNE BURNS

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_____
Holly R. Barnard

_____
Mark W. Williams

_____
Diane M. McKnight

Date_____

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline

Burns, Margaret Anne (M.A., Geography)

Hillslope Dissolved Organic Matter Transport and Transformation in a Semi-Arid Headwater Catchment

Thesis directed by Assistant Professor Holly R. Barnard

ABSTRACT

Dissolved organic matter (DOM) is a ubiquitous mixture of compounds formed from the degradation of both terrestrial and microbial materials. It is a key link across the terrestrial-aquatic interface in headwater catchments, and therefore is intimately linked with the hydrologic connectivity of the catchment to the stream. This study uses fluorescence spectroscopy and parallel factor analysis (PARAFAC) to evaluate the mobility of specific chemical constituents of DOM during snowmelt. Monitoring occurred on a daily basis within the shallow soil (10 – 25 cm depth) and the stream during snowmelt and was compared to approximately bi-monthly groundwater samples (~18 m depth). Results suggest that a transition occurred in the stream during snowmelt from DOM dominated by protein-like material to more humic-like material. This transition is indicative of an engagement of DOM originating from shallow soils during snowmelt. Dissolved organic carbon (DOC) normalization of these loadings suggest that the peak in DOC concentration seen in the stream is mainly controlled by the non-fluorescent fraction of DOM. This study identifies a humic-like component fluorophore in the soil and the stream that is traditionally seen only in soil DOM. We propose that the presence of this component in our stream samples during high flow in a small headwater catchment but not in larger systems during variable flow, suggests that this humic-like component is preferentially processed within the stream. These results indicate that shifts in hydrologic connectivity of

different watershed units to the stream are a major control on DOM export from the watershed and that DOM mobility is unique to its chemical composition.

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1. Introduction

Terrestrial dissolved organic matter (DOM) chemistry is one of the fundamental controls on the aquatic DOM chemistry in headwater streams (Wetzel 1992). The mobility of different chemical constituents of DOM during high soil saturation, specifically in snowmelt-driven catchments, is poorly understood. Due to its complex nature, identifying the chemical composition of DOM has proved difficult (Hedges et al. 2000). Fluorescence spectroscopy and parallel factor analysis (PARAFAC) are powerful tools that in combination, allow for the identification of the chemical fluorophores in the fluorescent fraction of the DOM (Coble et al. 1990, Stedmon et al. 2003, Cory & McKnight 2005, Murphy et al. 2008, Fellman et al. 2010). Fluorophores are operationally defined as the molecules in DOM that are able to absorb and re-emit light. Evaluating DOM chemistry using fluorescence spectroscopy in headwater catchments during high saturation provides an opportunity to characterize the temporal variability in DOM mobility (Miller & McKnight 2010, Inamdar et al. 2011b).

Studies in both rain-dominated (van Verseveld et al. 2008, Inamdar et al. 2011b) and snow-dominated (Hornberger et al. 1994, Hood et al. 2005) systems have shown increases in the input of highly aromatic and humic DOM fractions (typically from terrestrial sources) into the stream during solute flushing events (i.e. periods of high saturation and infiltration). This has been attributed to transitions in the dominant flowpath from groundwater sources to greater inputs from shallow soil water and surficial runoff. Understanding the hydrologic mechanisms causing these temporal changes in solute export is challenging, especially in snowmelt driven catchments, where asynchronous melting of the snowpack makes it difficult to identify the sources of solutes across the landscape (Boyer et al. 2000). Rain-dominated events cause more rapid, pulse-like water inputs, with greater input of DOM from surficial sources, (such as

throughfall and litter leachate) seen on the ascending limb of the hydrograph, and soil water inputs seen on the recession (Inamdar et al. 2011b). Results in alpine and subalpine snowmelt-driven catchments have shown similar patterns in DOC concentration, but with soil water as the primary control of DOC increases on the ascending limb of the hydrograph (Hornberger et al. 1994, Boyer et al. 1997, Hood et al. 2003). In high elevation, snowmelt-driven catchments, asynchronous snowmelt causes disproportionate contributions from different topographic positions within the landscape (Boyer et al. 2000). Despite differences between snowpack accumulation and distribution in both alpine and lower montane systems, the mechanism of asynchronous melt across the landscape is true at both scales. This mechanism is controlled by catchment-specific processes such as precipitation, topography, canopy cover, and soil development. Changes in climate are expected to cause dramatic changes in the timing of snowmelt in lower montane ecosystems (Clow 2010), making it especially important to understand the mechanisms behind solute and water delivery to the stream at these elevations.

During snowmelt, the amount of solute export to the stream from the hillslope is a result of the reactivity of these solutes along their flowpaths through the hillslope to the stream, as well as the hydrologic forcing via the amount of infiltration and melt. This is true for DOM and is caused by both the preferential retention of compounds (such as phenolic acids) and continuous mineralization by microbial communities (Kaiser & Kalbitz 2012). Compositional changes in DOM are dependent on various edaphic characteristics. Soils with high organic matter content are less likely to sorb DOM due to the occupation of cation exchange sites by soil organic matter (Kaiser & Zech 2000). Conversely, minerals such as aluminum and iron oxides that are found in the mineral soil have been shown to have a high sorption capacity, preferentially removing certain fractions of DOM from the mobile water (McKnight et al. 1992). Additionally, microbial

activity has been shown to be active throughout the soil, saprolite, and bedrock regardless of depth and soil composition (Buss et al. 2005, Eilers et al. 2012, Gabor et al. 2014). High soil heterotrophic activity, specifically under winter snowpacks, can increase the labile DOM pool during spring snowmelt (Brooks et al. 1999). The DOM remaining in the soil water is a result of the extent of these sorption processes in the soil. To date, studies of temporal changes in DOM chemistry have primarily focused on changes in surface waters (e.g. Miller & McKnight 2010, Jaffe et al. 2012). In addition, temporal changes in water soluble organic matter (WSOM) chemistry in soils has been studied (e.g. Corvasce et al. 2006, Hassouna et al. 2010, Gabor et al. 2014). Studies of the temporal dynamics of DOM in mobile soil water are limited (see Inamdar et al. 2011a).

Because of its reactivity within the soil, DOM in soil water is reflective of its origin and transport path. As a result, variation in stream DOM chemistry can be driven by catchment subsurface flowpaths. Reconciling temporally variable runoff chemistry with flowpath dynamics has been a focus over the last decade (Hruška et al. 2001, Bishop et al. 2004, Williams et al. 2006, Dawson et al. 2008). In this context, using fluorescence spectroscopy to study DOM composition can improve our overall understanding of these catchment hydrologic flow regimes, by providing an understanding of DOM mobility at the scale of chemical composition. While discrete sampling locations cannot explain the complete picture of catchment flowpaths, a greater understanding of the spatial variability of DOM across hillslopes can provide insights into the drivers of variable stream DOM chemistry (Hood et al. 2003, Inamdar et al. 2011b).

The objective of this study is to evaluate the temporal changes in DOM mobility during snowmelt in a mid-elevation headwater catchment in the Colorado Front Range. We focus on two main questions: 1) How does DOM composition vary spatially across two contrasting

hillslopes; and 2) What chemical fluorophores in DOM are mobilized in soil interstitial water during a snowmelt-driven flushing event? To answer these questions, we took a high frequency sampling approach and employed a combination of fluorescence spectroscopy and parallel factor analysis techniques.

2. Methods

2.1. Study site description

Our study was conducted in Gordon Gulch (2.6 km^2 and 2446 to 2737 m elevation), a headwater tributary to Boulder Creek, located within the Arapahoe National Forest in the Rocky Mountain Front Range of Colorado, USA (Figure 1). Gordon Gulch is part of the Boulder Creek Critical Zone Observatory (BcCZO), allowing this study to capitalize on existing infrastructure and records of catchment hydrology and biogeochemistry. The catchment receives approximately 520 mm of precipitation annually, with 60% in the form of snow (Cowie, 2010). It is characterized by distinct north- and south-facing slopes. Snow pack is highly variable in both space and time with more persistent snow pack on the north-facing slopes. The forests on the north-facing slopes are generally dominated by dense lodgepole pine (*Pinus contorta*) with little understory vegetation. South-facing slopes consist of low-density ponderosa pine (*Pinus ponderosa*) and grasses with frequent bedrock outcrops**.**

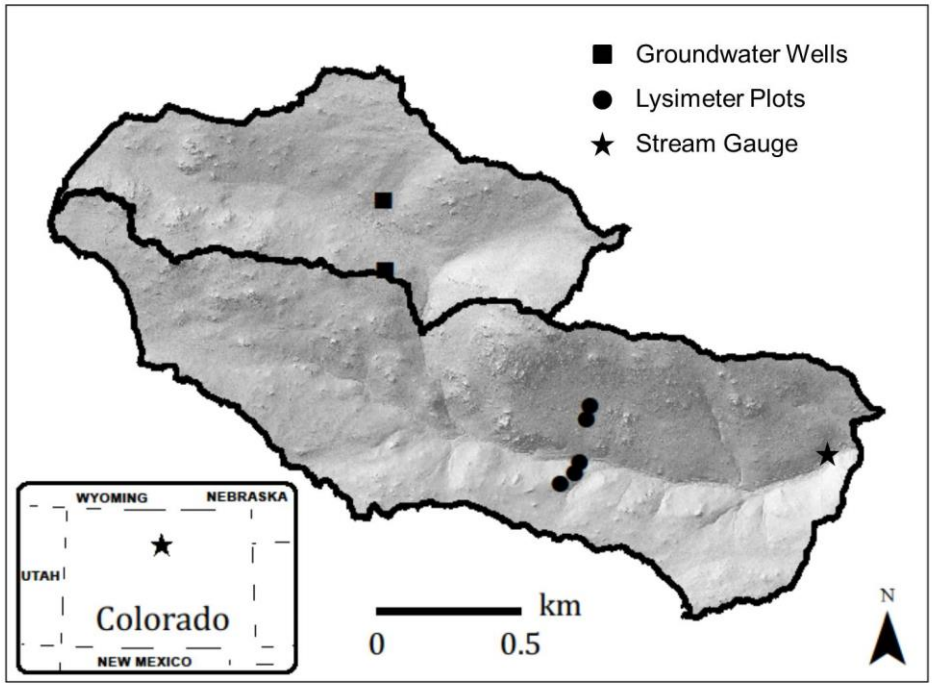

Figure 1: Site map of Gordon Gulch located within the Arapahoe National Forest. Lysimeter locations are marked with a circle and are located across a north- and south-facing transect. Groundwater well locations are marked with a square.

The north-facing slopes typically have deeper and more developed soil profiles in comparison to the south-facing slopes. Soils on the north-facing slope are from the Bullwark-Catamount families, primarily underlain by crystalline rock composed of biotite gneiss with sandy loam soils transitioning from cobbles to stones with depth (Natural Resources Conservation Service [NRCS] 2014). Previous work at Gordon Gulch has shown that north-facing slopes have deeper soils of approx. 50 cm, with a 5 cm O horizon above an A horizon (Gabor et al. 2014). Soils on the south-facing slope are of the Typic Haplustolls-Cathedral family, primarily composed of sandy loam to bedrock and are easily drained (NRCS, 2014). Soils on the south-facing slope are approximately 30-35 cm deep until saprolite (Hinckley et al.

2012), with 10 cm A horizons above mobile regolith (B/C horizons) (Gabor et al. 2014). Shallow seismic refraction (SSR) performed in Gordon Gulch in 2009 identified unweathered bedrock at depths of approximately 15 m on the north-facing slope and between 5 to 10 m on the south-facing slope (Befus et al. 2011). Between the base of the soil and unweathered bedrock is both saprolite (weak weathered rock) and weathered rock (strong, intact weathered rock) (Befus et al. 2011). The riparian zone on the transect is minimal, with no riparian zone on the south-facing side of the stream. The riparian zone on the north-facing side has no change in canopy cover or stand density in comparison to north-facing upland hillslopes. Riparian soils are thicker, approximately 170 cm depth to saprolite, with a 12 cm A horizon above mobile regolith (Gabor et al. 2014). Bulk density is lowest in the riparian zone with a bulk density of 1.15 g cm^{-3}. North- and south- facing slope bulk densities are similar at approximately 1.29 g cm^{-3}. Percent carbon was highest in the riparian zone (2.64%) followed by the north-facing slope (2.0%). Percent carbon on the south-facing slope was lower, at approximately 1.06%. Percent nitrogen follows the same trend with 0.15% in the riparian zone, 0.08% on the north-facing slope, and the lowest value of 0.05% on the south-facing slope. Values presented for bulk density, percent carbon, and percent nitrogen are averaged over a depth of 7-12 cm below the ground surface (Barnard et al. unpublished data). These depths were used because they were approximately at the same depth as the 10 cm lysimeter. No measurements were made at the 25 cm lysimeter depth for bulk density, percent carbon, and percent nitrogen. Microbial communities within Gordon Gulch are highly variable, showing highest microbial diversity at the soil surface with a subsequent decrease in depth. These changes in depth are thought to occur primarily due to edaphic factors such as changes in percent carbon and nitrogen as well as differences in soil pH and carbon quality (Eilers et al. 2012).

2.2. Sample collection

Tension lysimeters were installed to assess temporal and spatial variation in soil water DOM chemistry (Figure 1). Tension lysimeters were installed at approximately 10 cm and 25 cm depths, representing the soil-saprolite interface on the south-facing slope and within the mineral horizon on the north-facing slope. Lysimeters were made from PVC pipe with a porous ceramic cup (Soil Moisture Equipment Corp., Soil Water Samplers, Santa Barbara, CA). Three pairs of lysimeters were located on the north-facing slope and two pairs on the south-facing slope. Three piezometers were installed on the north-facing slope; one piezometer paired with each lysimeter site. Piezometers were installed at depths of approximately 1.2m, 1.3m, and 1.5m, from the high north-facing slope to the toe slope zone, respectively. The bottom 15.24 cm of each piezometer was screened to collect mobile water (Atlantic Screen & Manufacturing, Milton, DE).

Samples were collected daily from March 27 – May 19, 2013, excluding days where inclement weather inhibited site access (excluded dates: 4/9, 4/15-4/19, 4/23, and 5/1-5/2). Sampling stopped when two conditions were met: 1) seasonal snowmelt was complete on both slopes and 2) no water was present in any of the lysimeters 24 hrs after evacuation for two consecutive days. Lysimeters were evacuated to approximately 54,000 Pa 24 hours prior to each sampling (15cc Nalgene Repairable Hand Vacuum Pump ©). It is known that non-dissolved species in whole water samples can affect the fluorescent properties of DOM (Baker et al. 2007). Following the protocol of Cronan and Aiken, 1985, a 24 hour window between lysimeter evacuation and sample collection was considered an appropriate time duration, allowing enough time for water to collect while best preserving the DOM from degradation of non-dissolved species. A peristaltic pump was used to extract the water from the lysimeter (Global Water © SP200 Peristaltic Sampling Pump, College Station TX). The pump was flushed with ultrapure

deionized water between each sample. A few drops of sample were discarded and the remaining sample was collected. Amber glass bottles were acid-washed with 0.5 M HCl and combusted at 450°C for five hours before being stored with tinfoil coverings to ensure of no contamination. Samples were collected from all lysimeters and piezometers (if sample was present), as well as from the stream approximately 3 m upstream and downstream of the transect. Stream samples were collected as grab samples from the stream within the highest flow channel. All water samples were filtered within 24 hours of collection through 0.7 μ combusted glass fiber filters and stored in the dark at 4°C until analysis. On the north-facing slope, water was present in the lysimeters beginning on March 31, 2013 (DOY 90), concurrent with an increase in soil moisture to above 0.1 $cm^{-3}cm^{-3}$ at both the 5 cm and 25 cm depths. Snow samples were collected from the surface of the snowpack in approximately 10 cm x 10cm sections from the snow surface to a depth of 2 cm. Snow samples were collected throughout late March and April to capture spring storm events, occurring on 3/27, 4/12, 4/20, and 4/24.

Groundwater wells were located in Upper Gordon Gulch (Figure 1), with the north-facing well at 18.55 m depth and the south-facing well at 17.34 m depth, both located within the bedrock. The north-facing well was screened from 9.41 – 18.55 m and the south-facing well from 8.20-17.34 m (Anderson and Rock, 2013). Groundwater well samples were collected as part of the BcCZO routine sampling. Groundwater wells were pumped using a bladder pump (Geotech Bladder Pump) (Anderson and Rock, 2013). The pump was first rinsed with deionized water and subsequently, lowered into the well. Wells were pumped until conductivity and temperature stabilized to ensure fresh water. Combusted sample bottles were rinsed with sample three times and then filled and returned to the lab in a cooler until filtering. Samples were then filtered following the same protocol as the soil water samples. Due to limited sample size of

groundwater wells, groundwater values were averaged for wells located on north- and south-facing slopes.

2.3. Discharge, snowpack, and soil moisture

Stream discharge was measured at the base of the lower Gordon Gulch catchment (Figure 1). Discharge was measured using a pressure transducer (Solinst Model 3001 Levelogger Gold, Georgetown Ontario, Canada) monitored by the BcCZO (Anderson and Rock 2013). Discharge measurements were field-validated using manual salt conductivity tests and corrected using an annual rating curve (Anderson and Rock 2013). The BcCZO measured snowpack throughout the snowmelt season using time-lapse photography. Photos of snow poles were taken three times daily, at 8:00AM, 12:00PM, and 4:00PM (Anderson and Rock 2013). Here, we present daily averages for these three measurements at two snow poles. The approximate resolution for this approach is $\pm$ 2 cm. The south-facing snow pole is co-located with the high south-facing lysimeter plot in this study. Time-lapse cameras were not installed at the time of sampling on the mid or high north-facing slope; therefore, the values presented here are from a snow pole located in the riparian zone on the north-facing slope. This zone of the transect has greater total snowpack than the north-facing slope positions, but is representative of melt out on this slope. Missing values on the north-facing slope are due to poor visibility during snowfall. Monitoring on the south-facing slope was ceased on the last day of April.

Soil moisture was monitored at 10 minute intervals with CS616 soil moisture sensors and CR10x dataloggers throughout the season by the BcCZO (Campbell Scientific Inc., Logan, UT) (Anderson and Rock 2013). Sensors were installed at 5 cm and 20 cm depths within the soil on both the north- and south-facing slopes. Soil moisture sensors were co-located with the mid-slope south-facing plot used in this study, and the highest north-facing slope plot.

2.4. DOC concentration and DOM Spectroscopic analysis

An aliquot of each water sample was analyzed for DOC concentration using Pt-catalyzed high temperature combustion. Within run, and run to run precision was 1.33% relative standard deviation of standard duplicates with a detection limit of 0.07 mg C L^{-1} (following the methods of the Scientific Apparatus Makers Association) (Kiowa Laboratory, University of Colorado Boulder). Fluorescence spectroscopy was used to characterize the chemical composition of the DOM in the streamwater, lysimeter, piezometer, and groundwater samples. Samples were first run on an Agilent 8453 UV-vis spectrophotometer at an absorbance range of 190 to 1100 nm. Samples with an absorbance greater than 0.2 cm^{-1} at 254 nm were diluted to achieve an absorbance less than 0.2 cm^{-1} to allow for inner-filter corrections (Ohno 2002). A 4 ml room temperature aliquot of the diluted sample was then transferred to a 1 cm cell cuvette for fluorescence analysis (Horiba Jobin Yvon Fluoromax-3, Irvine, CA). Excitation emission matrices (EEMs) were created from excitation wavelengths 245 – 450 nm at every 10 nm and emission wavelengths 300 – 550 nm at every 2 nm with an integration time of 0.25 s and a slit width of 5 nm. Excitation emission matrices were corrected for variations in lamp intensity by normalizing the sample scan to the reference scan (ratio mode) (Cory et al. 2010). A blank using ultrapure deionized water was taken during each sample run and was subtracted from all EEMs after collection. Samples were then corrected for both instrument response and inner-filter effect prior to being Raman-normalized (Lawaetz and Stedmon,2009). We analyzed the fluorescence index of a Suwannee River Fulvic Acid standard during multiple runs of field samples as one measure of instrument accuracy (International Humic Substances Society). The Suwannee River Fulvic Acid sample was tested to have a fluorescence index of approximately 1.2, representing the terrestrial end of the fluorescence index scale.

2.5. PARAFAC model

After instrument correction and normalization, corrected EEMs were fit to an eight component parallel factor analysis model (PARAFAC) (Gabor 2013). This PARAFAC model identifies eight classes of fluorophores (components) with different chemical properties. It is a site-specific model created using intermittent spring water, soil leachates, and groundwater, all collected within Gordon Gulch (Table 1).

Table 1: Eight component Gordon Gulch PARAFAC model showing the description and location of each of the component fluorophores (Gabor 2013).

Component ID	Description	EEM Location
C1	Reduced quinone-like humic peak	ex. 250nm em. 500nm
C2	Reduced quinone-like humic peak	ex. 270nm & 340nm em. 440nm
C3	Oxidized quinone-like peak	ex. 240nm em. 425nm
C4	Unknown (possible clay scatter)	ex. 240nm and all emission wavelengths
C5	Soil humic-like peak	ex. 240nm & 325nm em. 400nm
C6	Unidentified humic-like peak	ex. 250nm em. 420nm
C7	Tyrosine-like protein peak	ex. 280nm 3m. 325nm
C8	Tryptophan-like protein peak	ex. 250nm em. 350nm

Parallel factor analysis is a statistical technique that decomposes the EEM into a combination of different fluorophores (each component represents a distinct fluorophore) (Stedmon et al. 2003). These fluorophores are measured in loadings, a unitless value that is directly proportional to the concentration of the fluorophore. Because the fluorescent fraction of DOM does not make up the entirety of the DOM, changes in the non-fluorescent fraction can affect the total DOM. Dissolved organic carbon makes up approximately 50% of the total DOM, and is therefore often used as a proxy for the concentration of the total DOM. In this paper, component loadings are presented as raw loadings to show quantitative changes in the fluorescent fraction of DOM,

while DOC normalized loadings are presented to show relative changes in the components compared to this larger DOM pool. This distinction is noted in all subsequent results. Percent protein was calculated using the PARAFAC component loading outputs. Components C7 and C8 represent the protein-like fluorescent fraction of the DOM. These two components were summed together and divided by the total fluorescence to identify the fraction of the fluorescent DOM that was protein-like (Fellman et al. 2009a).

2.6. Spectroscopic indices

Spectroscopic fluorescence indices were calculated using the corrected EEMs to identify changes in the chemical quality of the DOM. Fluorescence index (FI), the ratio of the emission intensity at 470 nm to the intensity at 520 nm at an excitation wavelength of 370 nm, was used to characterize the microbial (high FI) or terrestrial (low FI) nature of the DOM (McKnight et al. 2001). Humification index (HIX), the area under the peak from 435 – 480 nm emission divided by the area under the peak from 300-345 nm emission, was used to determine the degree of humification undergone by the organic matter. Greater HIX is indicative of more stable, aromatic compounds, typically from terrestrial, lignin-derived sources (Zsolnay et al. 1999, Ohno 2002). Freshness index (BIX), the ratio of the intensity at emission 380 nm divided by the maximum intensity between 420 – 435 nm emission at excitation 310 nm, was used as an indicator of the proportion of recently produced organic matter (typically of microbial origin), with a higher BIX indicating a greater contribution of recently produced organic matter (Parlanti et al. 2000, Wilson and Xenopoulous 2008). These indices represent different sections of the EEM, with the FI representing sections of the EEM with longer emission wavelengths, while BIX and HIX represent sections of the EEM with shorter emission wavelengths.

2.7. Data analysis

A one-way analysis of variance (ANOVA) in conjunction with a Tukey's honest significant difference (HSD) test was used to evaluate differences in spectroscopic indices and component loadings based on source location (groundwater, north-facing slope, south-facing slope, and riparian) and depth (10 cm lysimeter, 25 cm lysimeter, and piezometers). Lysimeter samples were then separated by aspect (north- and south-facing slopes and riparian) and two-tailed t-tests were then used to determine significant differences between component loadings in groundwater and soil water on the north-facing slope. North-facing slope soil water was used for comparisons with groundwater because of greater sample recovery, and previous research in the catchment has shown that matrix flow is more dominant on north-facing slopes (Hinckley et al. 2012). Linear and polynomial regression functions were used to evaluate the temporal changes in spectroscopic indices and raw component loadings in the stream throughout snowmelt. Pearson's correlation was used to determine the relationship between DOC normalized loadings and stream discharge. Because temporal trends were seen in the riparian piezometer, repeated measures ANOVA was used to assess differences seen between the riparian piezometer and the riparian lysimeter water. Differences were considered at the $\alpha = 0.05$ level of significance.

3. Results

3.1. Catchment hydrology and DOC dynamics during snowmelt

The ascending limb of the stream hydrograph initiated at approximately day of year (DOY) 110 (April 20, 2013) and continued to rise until its peak at DOY 130 (May 10, 2013) (Figure 2A). Catchment groundwater table on the north-facing slope rose synchronously with the stream hydrograph, peaking approximately 3 days after discharge (DOY 133) (Figure 2A). There was a concurrent increase in stream DOC concentration along the ascending limb of the stream hydrograph from approximately 3.5 mg C L^{-1} to 8 mg C L^{-1}, also reaching peak concentration on

DOY 130 (Figure 2B). Despite a rise in the water table of the groundwater, groundwater DOC concentrations were stable throughout snowmelt at 1-2 mg C L^{-1} (n=7). Soil water DOC concentration was more variable but there was no temporal trend throughout snowmelt ($p = 0.8$; n=74). Typical values of soil water DOC concentration ranged from approximately 30 – 35 mg C L^{-1} on both the north- and south-facing slopes. The DOC concentration did not change temporally in the riparian piezometer ($p=0.2$) or in the north-facing piezometer ($p=0.06$). No significant differences in DOC concentration were found across aspects ($p=0.66$), between the north-facing slope and the riparian zone ($p=0.37$), or between the south-facing slope and the riparian zone ($p=0.99$). The input of dissolved organic carbon concentration from snowpack was minimal, ranging from 0.33 – 1.78 mg C L^{-1} throughout the melt season.

Soil moisture at 5 cm on the south-facing slope averaged 0.08 $cm^{-3}cm^{-3}$ with a peak water content of 0.125 cm^3cm^{-3} on DOY 129 (May 9). At a depth of approximately 20 cm, volumetric water content averaged 0.14 cm^3cm^{-3} during the study period, with a peak of 0.18 $cm^{-3}cm^{-3}$ on DOY 129 (May 9). Soil moisture was greater on the north-facing slope (Figure 3A). Volumetric water content averaged 0.13 cm^3cm^{-3} at a 5 cm depth, with a peak on DOY 128 (May 8). At a depth of approximately 20 cm, average soil moisture was 0.16 cm^3cm^{-3} with a peak of 0.21 cm^3cm^{-3} on DOY 128 (May 8) (Figure 3A). Soil moisture on the north-facing slope during baseflow conditions (late September – early October) is approximately 0.06 cm^3cm^{-3} at both the 5 cm and 25 cm depths, respectively. Soil moisture on the south-facing slope during baseflow conditions is approximately 0.01 cm^3cm^{-3} at the 5 cm depth and 0.05 cm^3cm^{-3} at the 25 cm depth. Sample collection on the south-facing slope was sparse, with a few limited samples at the beginning of snowmelt in the 10cm lysimeter, and samples in the 25 cm lysimeter only occurring in late April. Soil moisture was approximately 0.5 cm^3cm^{-3} at the 5 cm depth and above 0.1

cm^3cm^{-3} at the 25 cm depth when the first sample was recovered (DOY 90). Because the presence of water in the lysimeters only occurred during high soil saturation conditions during snowmelt, we suggest that the DOM used in this study is representative of the more mobile classes of DOM in the soil matrix; not the organic matter tightly bound to soil matrix surfaces (Six et al. 2004). Snowpack was lower on the south-facing slope, with a maximum snowpack of approximately 40 cm, compared to approximately 80 cm on the north-facing slope (Figure 3B). Snowpack melted out much more frequently on the south-facing slope (Figure 3B).

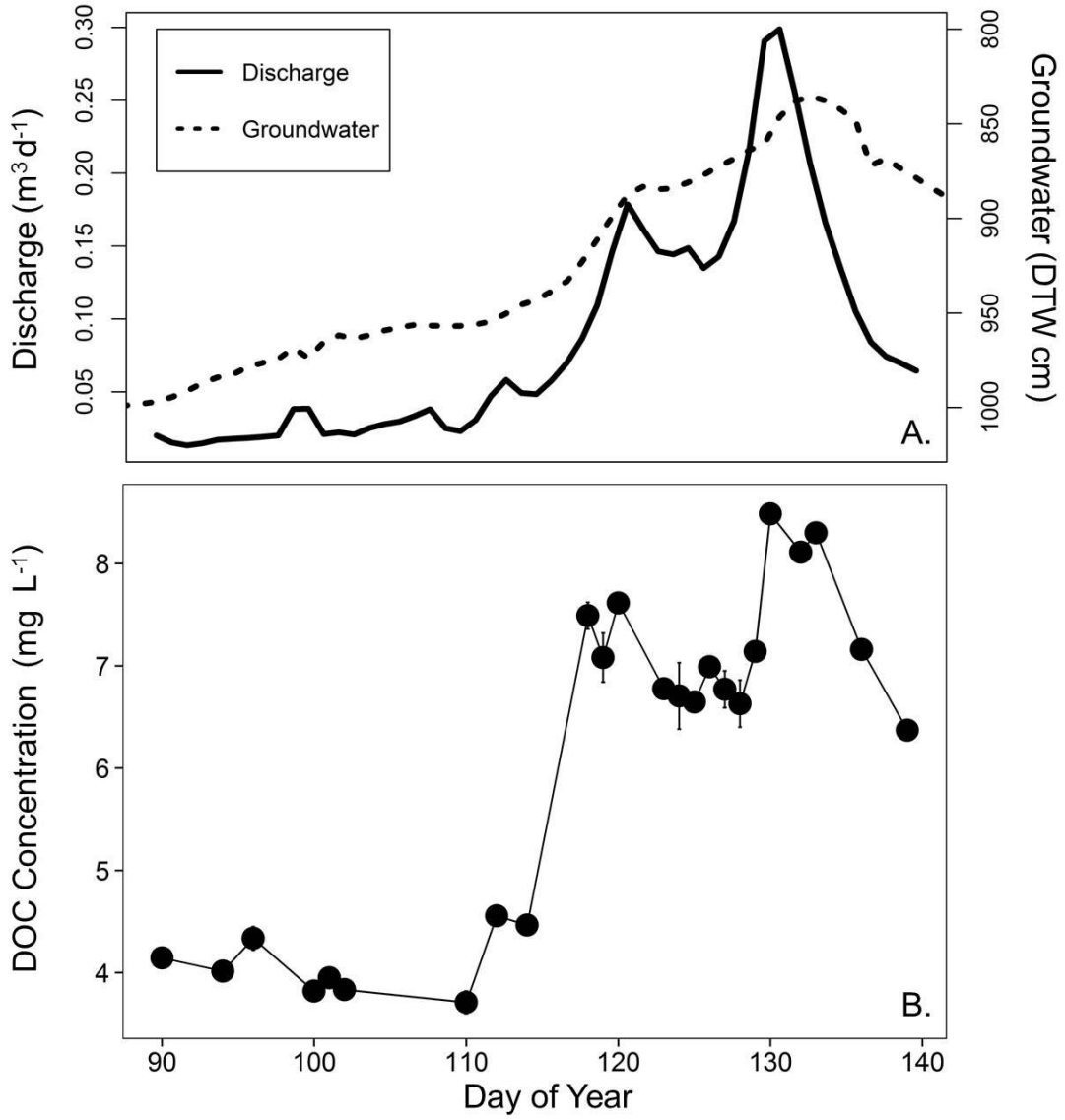

Figure 2: A) Stream discharge (m^3 d^{-1}) and depth to water (DTW) in the north-facing groundwater well (cm). B) Dissolved organic carbon concentration in the stream throughout the snowmelt season. Error bars represent $\pm$ SE for the average between the two stream collection sites.

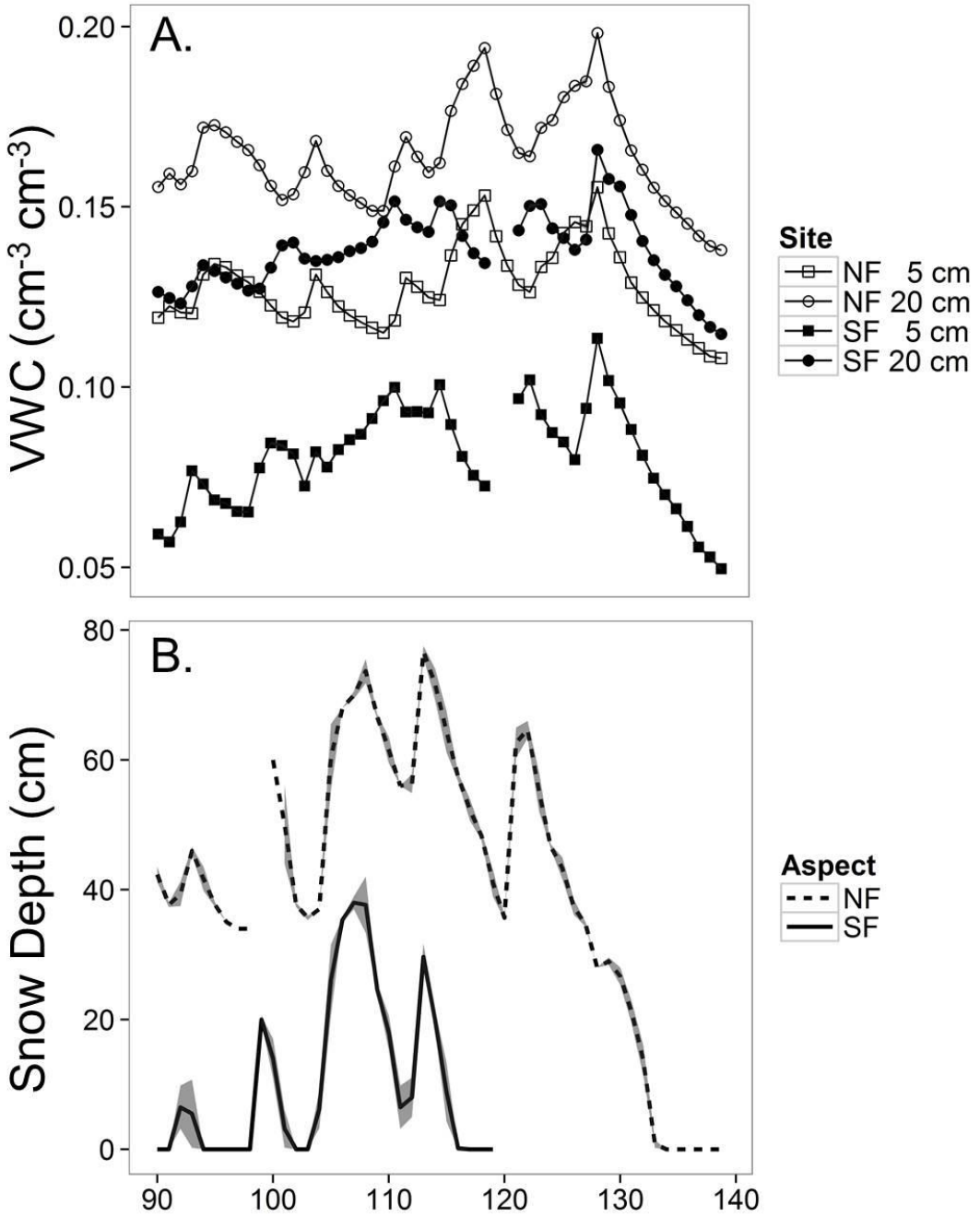

Figure 3: A) Soil moisture at the 5 cm and 20 cm depths on the north-facing (NF) and south-facing (SF) plots and B) Snow depth measured on the north- and south-facing slopes throughout the snowmelt season. Depths are daily averages from measurements at 8:00AM, 12:00PM, and 4:00PM. Broken lines are due to missing data. Grey shading on both figures indicates $\pm$ SE. VWC = volumetric water content.

3.2. PARAFAC Component Loadings

No significant differences in component loadings were found between lysimeter samples from the 10 cm and the 25 cm depths, therefore the depths from each site were aggregated ($p>0.05$). The 10 cm lysimeter on the high north-facing slope had significantly greater contributions of humic-like components C5 and C6 than the 10 cm lysimeter on the mid-slope (C5: $p<0.01$, C6: $p=0.02$). This was not seen between the 25 cm depths at each plot, nor between the 10 cm and 25 cm depths at each of these two north-facing plots and therefore, suggests that this difference was minimal. Consequently, the mid-slope and upper north-facing sites were averaged together for comparisons to groundwater and to the stream. Humic-like components C2 and C5 were significantly different between slopes, with higher values of both components on the north-facing slope ($p<0.05$). This suggests more humic-like material on the north-facing slope. The north-facing slope was used for all comparisons with groundwater because the number of samples was much greater and previous research suggests that soil matrix flow is a more dominant mechanism on the north-facing slope than the south-facing slope (Hinckley et al. 2012). Sample recovery on the south-facing slope was sparse, with no samples obtained from either the 10 cm or 25 cm depths on the high south-facing slope and a total of only 24 samples from the mid-slope location (occurring on 17 out of a total of 45 sampling days). No significant temporal trends were found in component loadings in either groundwater well ($p>0.05$). Furthermore, no significant differences in component loadings were found between the two groundwater wells ($p>0.05$), so all samples from the two wells were averaged together over the snowmelt season. However, it is important to take into consideration the small number of groundwater samples ($n=7$) used in this study.

No temporal trends were found in component loadings from lysimeter samples therefore, loadings from the entire snowmelt season on the north-facing slope were averaged (n=54) (hereafter 'soil water' is used to indicate these averaged north-facing samples). Raw loadings in the soil water were significantly higher than groundwater for humic-like components (C1, C2, and, C6), the oxidized quinone-like component (C3), and the tryptophan-like component (C8) ($p<0.01$ for all comparisons) (Table 2). Groundwater had a significantly higher raw loading of the tyrosine-like component (C7) ($p<0.01$) (Table 2). The soil humic-like component (C5) showed no significant difference between soil water and groundwater ($p=0.99$) (Table 2). In the stream, raw loadings for humic-like component C1 and oxidized quinone-like component C3 increased throughout snowmelt following a nonlinear trend ($p<0.01$, R^2 = 0.72 and 0.75, respectively) (Figure 4A). Humic-like components C2, and C6 increased significantly, and protein-like component C7 decreased significantly following a linear trend ($p<0.01$, R^2 = 0.53, 0.22, and 0.83, respectively) (Figure 4B). Components C5 and C8 showed no significant temporal trend ($p>0.05$) (data not shown). The north-facing piezometer showed a significant decrease in soil humic-like component (C5) between DOY 119 and DOY 130 ($p<0.01$). No other components changed temporally in the north-facing piezometer ($p>0.05$), suggesting that the sorption of component C5 is potentially greater than all other components. This could also be a limitation of the small sample size and date range of this piezometer. In the riparian piezometer, humic-like components (C1, C2, and C5) and oxidized quinone-like component C3 increased during snowmelt ($p<0.05$) and tyrosine-like component C7 decreased significantly ($p<0.01$). Using repeated measures ANOVA to account for temporal changes in the piezometer, significant differences ($p<0.05$) were seen in all components between the riparian piezometer and lysimeters

except tryptophan-like component C8. Sample size (n=8) for the riparian piezometer could be limiting these analyses and therefore, must be considered.

Table 2: Mean and standard error (SE) for all components (excluding C4) for both the raw, and DOC normalized loadings in the groundwater and soil water. Significant differences between groundwater and soil water are marked with an asterisk (*).

	Raw Loading		**DOC Normalized Loading**	
Component	Groundwater	Soil Water	Groundwater	Soil Water
C1	0.06(0.01)*	0.39(0.03)	0.30(0.07)*	0.013(<0.01)
C2	0.08(0.01)*	0.25(0.01)	0.41(0.07)*	0.01(<0.01)
C3	0.11(0.03)*	0.47(0.03)	0.51(0.17)*	0.016(<0.01)
C5	0.10(0.02)	0.13(0.01)	0.51(0.12)*	0.009(0.01)
C6	0.04(0.01)*	0.12(0.01)	0.17(0.06)	0.005(<0.01)
C7	0.49(0.05)*	0.06(0.01)	2.26(0.15)*	0.009(0.01)
C8	0.01(<0.01)*	0.04(0.02)	0.07(0.03)	0.014(0.01)

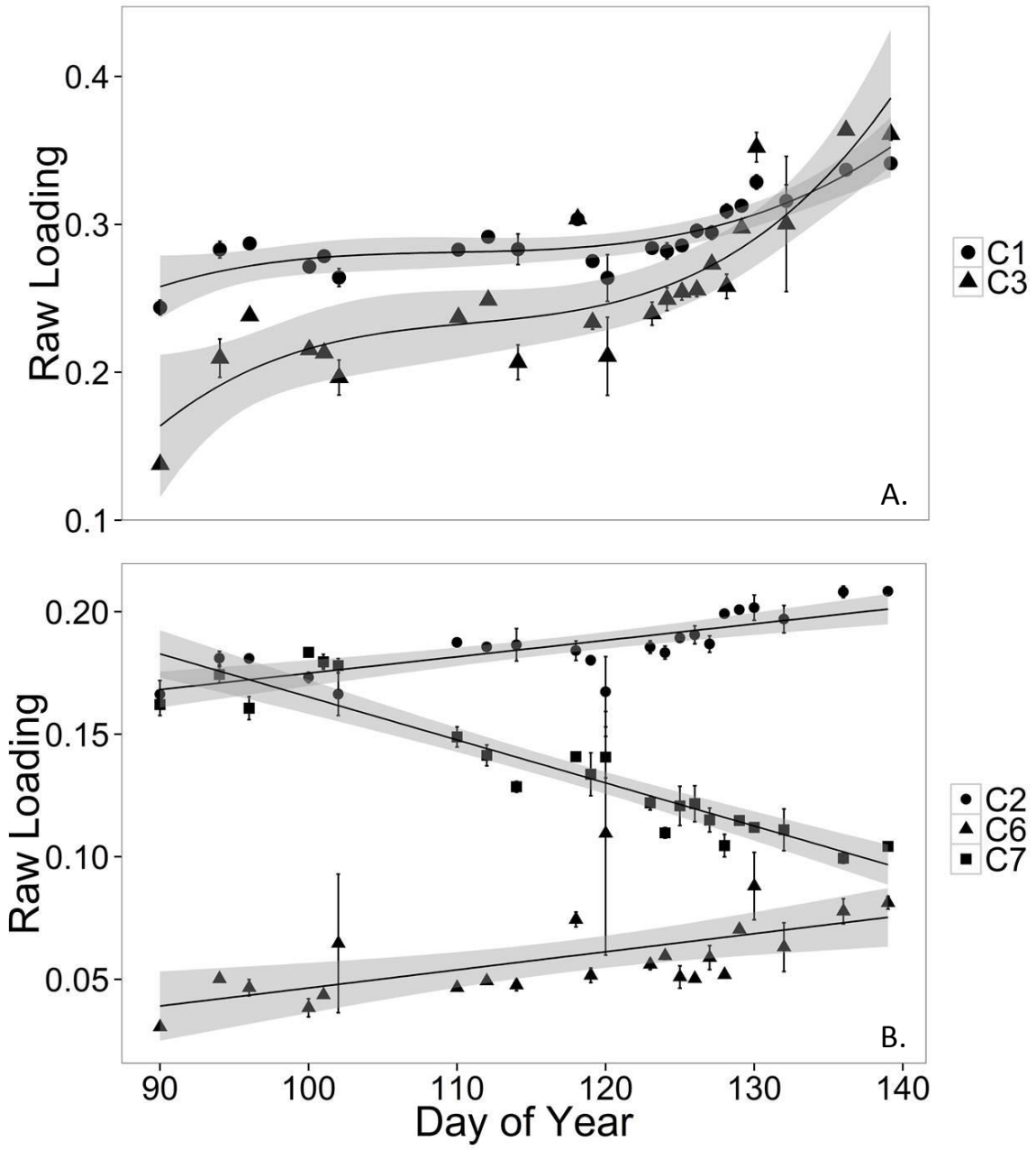

Figure 4: Raw component loadings in the stream over the snowmelt season for A) components C1 and C3, and B) C2, C6, and C7. All regressions are significant ($p<0.05$). Components C1, C2, and C6 represent humic material. Component C3 is an oxidized quinone-like moiety and component C7 is a tyrosine-like protein. Grey shading represents the 95% confidence interval around the smoothing line.

We normalized the raw loadings to the DOC concentration to compare any relative changes in the fluorescent DOM to the total DOC pool. After DOC normalization, groundwater loadings were significantly higher than soil water for the humic-like components (C1, C2, and, C5), the oxidized quinone-like component (C3), and the tyrosine-like component (C7) ($p<0.05$) (Table 2). This is caused by a high DOC concentration in the soil water which reduces the magnitude of the soil water loadings. After DOC normalization, components C1, C2, C3, C5, C7, and C8 in the stream were significantly inversely correlated with stream discharge ($p<0.01$). When DOC normalized, tyrosine-like component (C7) actually increases through time ($p=0.02$) in the north-facing piezometer. It is most likely that this is a function of limited sample size and date range. No other DOC normalized components changed temporally in the north-facing piezometer ($p>0.05$). When normalized to the DOC concentration, the riparian well shows significant increases in humic-like components C1, and C2, as well as oxidized quinone-like component (C3) and tryptophan-like component (C8) ($p<0.05$ for all comparisons). Component C4 is thought to represent particulate matter scattering (Gabor 2013), and is not representative of a true organic matter fluorophore. Consequently, it was not included in component loading analyses.

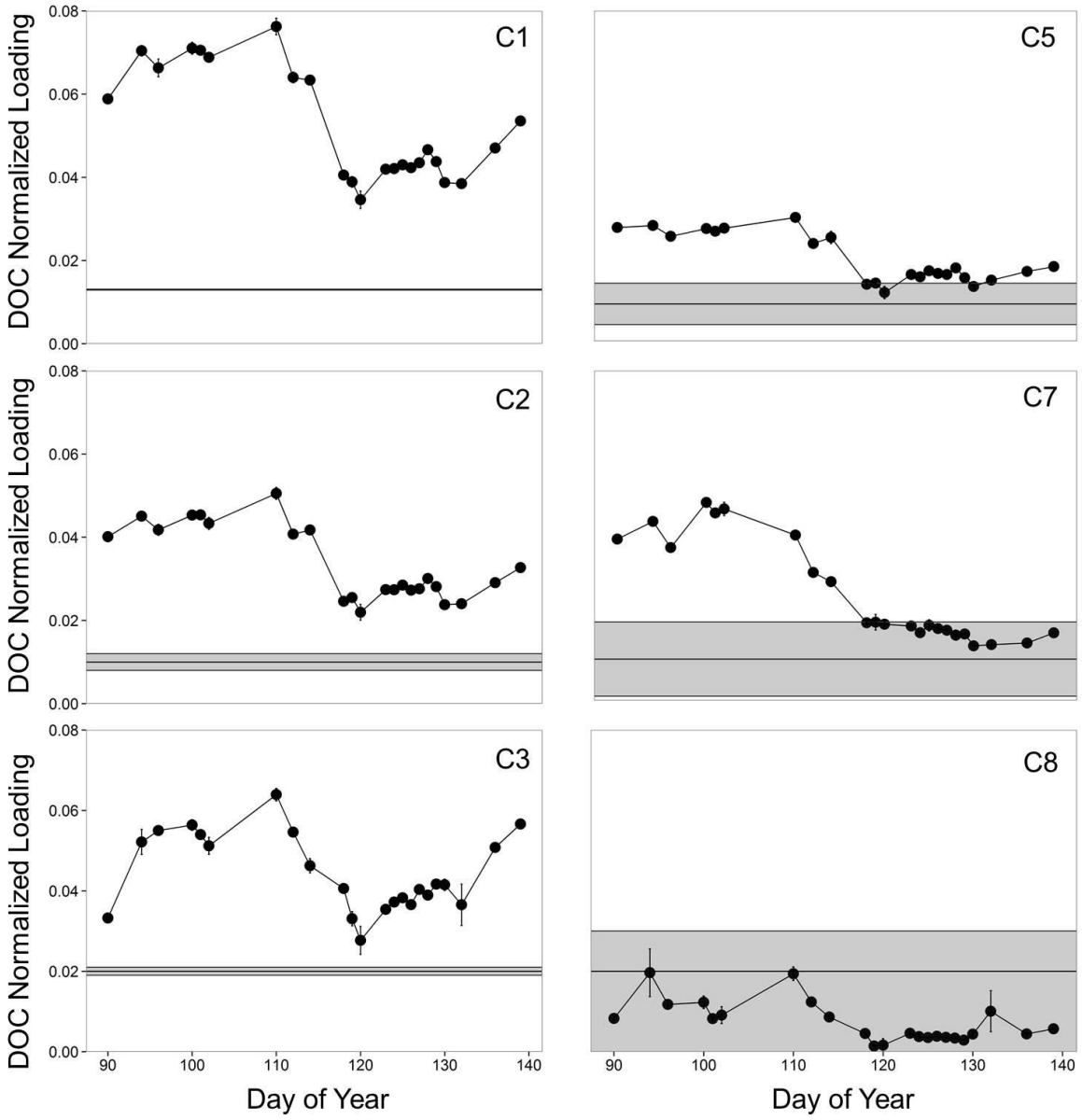

Figure 5: DOC normalized loadings of components C1, C2, C3, C5, C7, and C8 in the stream during snowmelt. Horizontal black line indicates the average DOC normalized loading in the soil water for that component and the shaded grey box is $\pm$ 1 SE. Groundwater loadings for all components is greater than the maximum stream loading. All regressions are significant ($p<0.05$). Error bars represent $\pm$ SE for the two stream sample site locations.

3.3. Spectroscopic Indices

No temporal trends were found in the spectroscopic indices of the soil water, groundwater, or the north-facing piezometer ($p>0.05$). Percent protein was not significantly different between aspects ($p=0.94$), the north-facing slope and the riparian zone ($p=0.79$) or between the south-facing slope and the riparian zone ($p=0.99$) (Table 3). The north-facing piezometer was not significantly different than all soil water sites ($p>0.05$) (Table 3). All lysimeter locations and the north-facing piezometer had significantly less protein than the groundwater samples ($p<0.01$) (Table 3). The humification index was marginally different between aspects ($p=0.05$), with south-facing slopes having a higher HIX than north-facing slopes. The humification index was significantly higher ($p<0.01$) in the riparian zone than on the north-facing slope, indicating larger, more aromatic compounds in the riparian zone (Table 3). The north-facing piezometer had a significantly lower HIX than the north-facing slope ($p<0.01$), south-facing slope ($p<0.01$), and riparian zone ($p<0.01$). There was no aspect difference seen with the BIX index ($p=0.97$) or between either slope and the riparian zone (north: $p=0.10$, south: $p=0.38$), indicating that there is no difference in the age of the organic matter across the two slopes (Table 3). The north-facing piezometer was not significantly different than soil water at all locations (north: $p=1.0$, south: $p=0.99$, riparian: $p=0.81$). Significant differences seen between the 25 cm lysimeter and the piezometer in the riparian zone, suggest that this soil water in the upper 25 cm is not mixed with depth in the riparian zone. The fluorescence index was significantly different between the north- and south-facing aspects ($p<0.05$), suggesting relatively greater microbial input on the north-facing slope (higher FI) (Table 3). The riparian zone had a significantly lower FI than the north-facing slope ($p<0.05$), most likely due to increased terrestrial material in this zone. The north-facing slope piezometer was not

significantly different than all lysimeter locations ($p>0.05$ for all locations). The mid-slope piezometer did not have water table development through snowmelt, therefore, no samples were available from this piezometer.

Table 3: Mean and standard error (SE) for the humification, freshness, and fluorescence indices at various watershed positions. Significance between sites is noted by the corresponding letters ($p<0.05$).

Location	**Protein (%)**	**HIX**	**BIX**	**FI**
North-Facing Slope	8.20 (1.31) a	10.96 (0.54) a	0.47 (0.01) a	1.39 (0.01) a
South-Facing Slope	5.18 (0.95) a	13.74 (1.35) b	0.47 (0.01) a	1.29 (0.01) b
Riparian	3.73 (0.26) a	15.68 (1.71) b	0.43 (0.01) a	1.31 (0.01) b
NF Piezometer	7.39 (0.36) ab	5.76 (0.33) c	0.48 (0.01) a	1.37 (0.01) ab
Groundwater	58.47 (4.82) c	2.60 (0.60) c	0.88 (0.04) c	1.58 (0.02) d
Riparian Piezometer	min: 10.59 max: 28.76	min: 1.08 max: 4.02	min: 0.59 max: 0.79	min: 1.22 max: 1.39
Stream	min: 11.21 max: 25.50	min: 1.80 max: 3.85	min: 0.55 max: 0.68	min: 1.29 max: 1.36

Spectroscopic indices in the stream changed temporally. Percent protein in the stream decreased from approximately 25% to 11%, indicating a potential shift from microbial DOM (high percent protein) during the onset of snowmelt to more terrestrial DOM (low percent protein) during peak snowmelt ($p<0.01$) (Figure 6A). The stream exhibited a significant increase in HIX ($p<0.01$) indicating a shift towards more humified organic matter material during snowmelt (Figure 6B). Concurrently, a significant decrease in BIX was seen in the stream during snowmelt ($p<0.01$), indicating a decrease in recently produced, microbial DOM (Figure 6C). The

FI increased significantly in the stream, indicating increased input from microbial production ($p<0.01$) (Figure 6D). Despite this increase, the entire range of the FI (1.29 – 1.36) is relatively high in terrestrial material compared to microbial materials. Fluorescence index typically ranges from 1.2 – 1.9, with lower values indicating relatively greater terrestrial input and higher values indicating greater microbial input (McKnight et al. 2001). Temporal changes were observed in the spectroscopic indices of the riparian piezometer (n=13, DOY range: 119-130). The HIX increased significantly through time ($p<0.01$), and the percent protein decreased through time ($p<0.01$). The fluorescence index in the riparian piezometer did not change significantly through time ($p=0.06$). These trends are consistent with the temporal trends seen in the stream, indicating a decrease in the humification of the material, and an increase in the protein-like material.

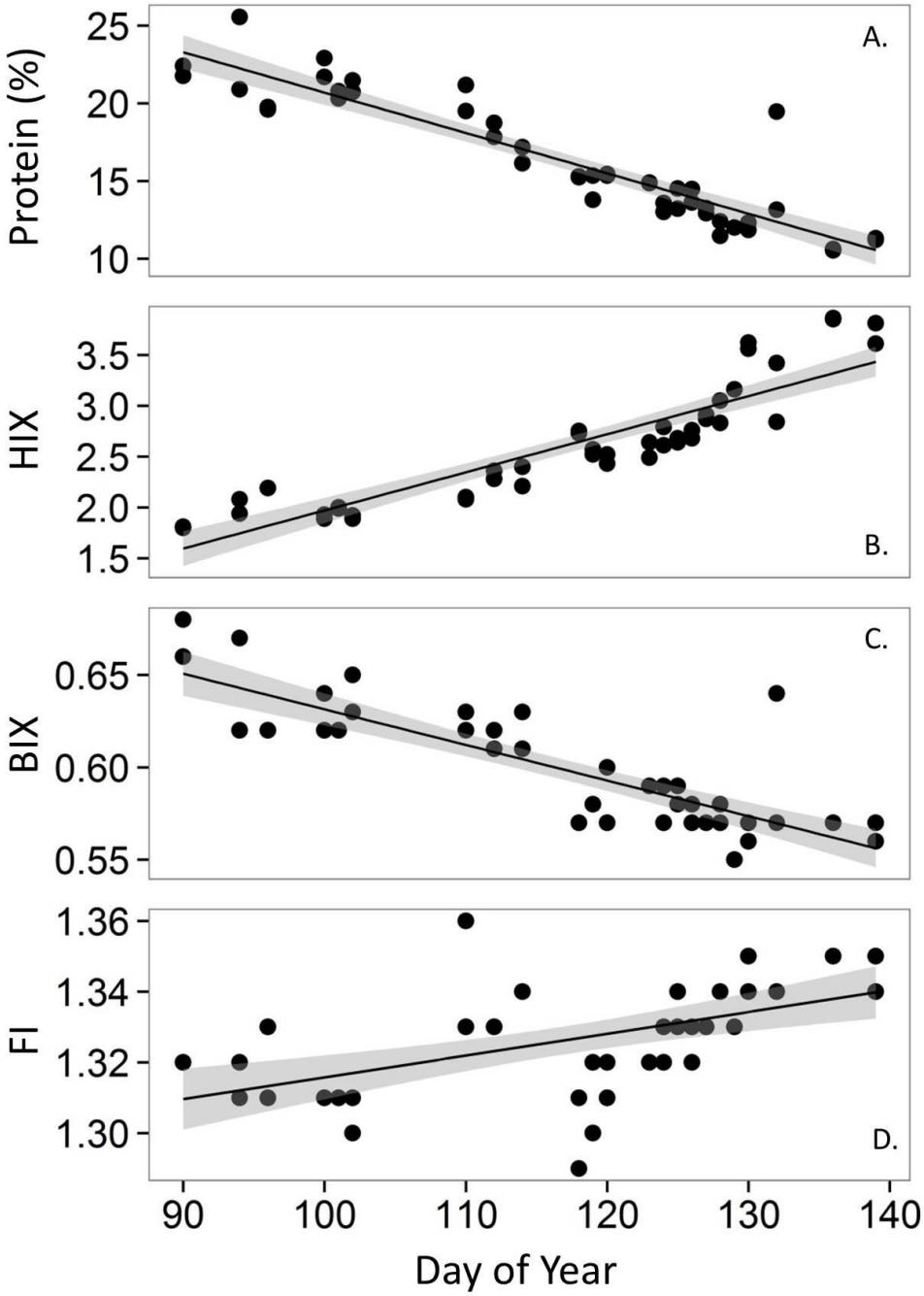

Figure 6: Linear regressions showing the change in A) Percent Protein (R^2 = 0.83) B) Humification Index (R^2 = 0.80), C) Freshness Index (R^2 = 0.69), and D) Fluorescence Index (R^2 = 0.29) in the stream during the 2013 snowmelt season. All regressions are significant ($p<0.05$). Grey shading represents the 95% confidence interval around the linear smoothing line.

4. Discussion

Soil water DOM is a major allocthonous contributor to stream water DOM during snowmelt (Boyer et al. 1997, Pacific et al. 2010). Our study provides insight into our understanding of temporal variability in the fluorescent fraction of DOM during snowmelt in a montane, headwater catchment. Using DOC concentration and spectroscopic indices, previous work at higher elevations in the Rocky Mountains demonstrated an increase in the input of shallow water sources to the stream during snowmelt (Hornberger et al. 1994, Hood et al. 2005). Our study confirms that this process is true at the hillslope scale at lower elevations within the montane zone, and that this shift in sources can be seen in the specific chemical fluorophores using PARAFAC analysis. We suggest that mobility of the humic fraction and its appearance in the stream during snowmelt is a result of increased hydrological connectivity between shallow soil sources and the stream. The presence of these humic materials in the soil water but to a much lesser extent in groundwater, suggests that it is terrestrially sourced, primarily composed of lignins, tannins, polyphenols, and melanins (Green and Blough 1994, Del Vecchio and Blough 2004), as well as quinone moieties (Cory and McKnight 2005).

4.1. Allocthonous DOM chemistry

In our study, soil water DOM is high in quinone-like molecules and humic substances, while deeper groundwater reflects a relatively greater contribution of protein-like DOM. Previous research in Gordon Gulch noted high contributions of humic substances in the water soluble organic matter (WSOM) in shallow soils (Gabor et al. 2014). The presence of these substances in the soil water suggests that these terrestrially-derived humic substances found in the soil are readily mobilized. However, depth-dependent trends found within the first few meters in the WSOM, such as a decrease in aromaticity, (Gabor et al. 2014) are not present in the

soil water. The similarity in the DOM composition between the lysimeter soil water and the north-facing hillslope piezometer suggests that the soil water is well-mixed down to our monitoring depth of 1.2 m within the saprolite. However, only one hillslope piezometer location produced water during snowmelt and therefore, these interpretations are limited to this location. Groundwater wells within the bedrock, at a depth of approximately 18 m below the ground surface, have low total DOC concentrations and are less humic in nature. This suggests that DOM in mobile waters is different on the order of several meters on the hillslope, not within the shallow mobile regolith. Deeper groundwater sources have been shown to be lower in chemical complexity and molecular weight, due to microbial processing of higher molecular weight compounds (McDowell 1982), as well as preferential adsorption of hydrophobic high molecular weight compounds by minerals (Qualls and Haines 1992). During our study, the water table rose during snowmelt, peaking three days after discharge (DOY 133). This suggests that vertical infiltration is occurring rapidly, however, the low DOC concentration and change in the chemical character of the DOM between soil water and groundwater provide evidence that this DOM changes rapidly along the vertical flowpath. North-facing slopes at Gordon Gulch have thick layers of saprolite and weathered bedrock, extending from the bottom of the soil profile (approximately 50 cm) to unweathered bedrock (approximately 15 m) (Befus et al. 2011). Vertical flowpaths to groundwater intersect this thick region of weathered saprolite and bedrock. Biotite has been identified as an important mineral at Gordon Gulch (Kelly 2012) and is known to cause microfractures in bedrock due to its expansion during hydration (Isherwood and Street 1976). This mechanism could be responsible for both the rapid vertical transport of water to depth and the exposure to fresh mineral surfaces within the bedrock and saprolite. Microbial activity has previously been documented in crystalline bedrock, and can impact the properties of

carbon (Pedersen et al. 1991). Therefore, the degradation of DOM at our study site could also be indicative of rapid microbial processing of DOM at depth.

4.2. Temporal trends in PARAFAC components

4.2.1. Raw Loadings

Temporal changes in stream chemistry are consistent with previous studies in Colorado, showing an increase of humic substances during snowmelt in an alpine stream (Hood et al. 2003) and an alpine lake (Miller et al. 2009). We observed an increase in the raw loadings of the humic-like components (C1, C2, and C6). Fulvic acids derived from allocthonous inputs, (a large contributor to humic materials during snowmelt (Miller & McKnight 2010)) can fractionate in the stream due to the presence of iron and aluminum oxides (McKnight et al. 1992). This process can remove molecules containing greater aromatic content and carboxylic acid functional groups due to preferential sorbtion onto oxide surfaces, a process known as ligand-exchange complexation (McKnight et al. 1992). Therefore, depending on the soil composition, certain DOM molecules can be preferentially sorbed onto soil particles, removing these molecules from the soil water. Humic-like components typically contain chemical moieties that are easily sorbed to the soil (McKnight et al. 1992). Our increase in the export of humic material to the stream during snowmelt indicates that hydrologic transport through shallow soils may be overwhelming the sorption capacity of the soil and is not allowing enough contact time to remove these humic-like molecules. It is also possible that these soils have few oxide surfaces, and therefore, have more limited sorption capacity. This lack of sorption can be seen specifically with our humic-like component C1, which corresponds to a semi-quinone component (SQ1), identified in the 2005 Cory & McKnight model. They showed that this terrestrially-derived humic component was positively correlated with aromatic carbon (Cory & McKnight, 2005). Therefore, our observed

increase in C1 in the stream could be indicative of more aromatic carbon delivery to the stream. Despite rapid sorption rates of hydrophobic aromatic molecules to soil surfaces (McKnight et al. 1992), we still see an increase in C1 in the stream throughout snowmelt. The nonlinear increase in components C1 and C3 suggests that the sorption processes of these components differs from the other components. This could also be a result of the location of these components within the soil profile. This further indicates that during snowmelt, the sorption capacity of the soils is low, allowing the transport of terrestrially-derived hydrophobic molecules to the stream.

Component C5 is the only humic-like component that does not demonstrate an increase throughout snowmelt in the stream. This component has been found in a limited number of studies and is unique to the soil matrix (Ohno et al. 2009, Gabor et al. 2014). Its presence in soil water and stream water during our study suggests that it is initially mobilized and exported to the stream. Raw loadings of component C5 are not significantly different between groundwater and soil water. Therefore, raw loadings of component C5 do not reflect changes in the hydrologic connectivity of catchment sources to the stream. Contrary to our findings, this component has not been found in stream samples using PARAFAC analysis (eg. Cory & McKnight 2005). We speculate that this component is seen in our stream samples because sampling occurred during periods of high connectivity of the hillslopes to the stream. Its absence in larger streams during variable periods of flow (Cory & McKnight 2005), suggests that this component is preferentially and rapidly processed in the stream, through in-stream processes such as photodegradation, microbial activity, and hyporheic exchange. We have seen this rapid processing at Gordon Gulch, where tracer experiments have shown rapid loss of DOM in the stream during low flow periods, likely as a result of these processes (Gabor 2013).

The temporal decrease in the protein-like fraction of the fluorescent DOM in the stream supports our hypothesis that shallow soil water becomes hydrologically connected to the stream during snowmelt. Our observed decrease in the percent protein in the stream during flushing is consistent with other studies (Nguyen et al. 2010, Inamdar et al. 2011b) and is thought to be a consequence of a transition away from groundwater sources during snowmelt. This temporal decrease in total percent protein is controlled by the temporal decrease in the tyrosine-like component (C7) and not the tryptophan-like component (C8). Our soil water has significantly lower tyrosine-like DOM and significantly higher tryptophan-like DOM than groundwater. A transition in the stream towards less tyrosine-like DOM supports an increase in soil water to the stream during snowmelt. It is also important to consider that the transfer of energy from tyrosine to tryptophan can cause quenching of the fluorescence of tyrosine when tryptophan fluorescence is high (Stedmon & Markager 2005). Therefore, our significantly higher tryptophan-like DOM in the soil water could be quenching the fluorescence signal of tyrosine, creating an illusion towards lower tyrosine-like DOM in the stream. However, this mechanism seems unlikely because for this to occur, the tyrosine and tryptophan must be on the same molecule. The inclusion of both proteins onto one molecule increases the molecular weight of the molecule and consequently increases its affinity for sorption onto free cation exchange sites on soil particles. It is therefore more likely to be sorbed to the soil quickly and less likely to be present in the mobile soil water. However, without further isolation of the DOM, we can only speculate as to the molecular character of the DOM.

A shift towards less protein in the stream has implications for DOM biodegradability for autocthonous heterotrophs. A higher percent protein corresponds to more biodegradable DOM (Fellman et al. 2009b) and has a higher microbial uptake rate (Fellman et al. 2009a). Our results

suggest that during snowmelt, the percent protein in the stream decreases. Therefore, this labile source of DOM (Fellman et al. 2009b) becomes less available for heterotrophic processing in the stream. However, lability is difficult to quantify solely from generalized classes of DOM because it is influenced by properties such as the molecular structure and stoichiometry of the specific molecules making up these classes (Findlay & Sinsabaugh 2003). For example, despite their typically complex chemical structure and recalcitrant nature, some humic substances are biodegradable and provide important nutrient sources for in-stream heterotrophs (Moran and Hodson 1990). Our identified soil humic-like component C5 could be of this class. Heterotrophic processing can also depend on the duration of the nutrient input. During episodic events, such as snowmelt, it is possible that stream heterotrophs need time to adapt to large nutrient inputs and are not necessarily able to process solutes as quickly (Fellman et al. 2009a). There is no doubt that, in some capacity, these processes are all occurring in the stream at different times and spaces. Therefore, while a decrease in the percent protein removes this potentially labile solute source, it cannot be considered solely responsible for nutrient availability in the stream.

4.2.2. DOC Normalized Loadings

While the fluorescent fraction of DOM operates as a tracer showing the large influx of shallow, terrestrial DOM to the stream, it is important to consider that other sources of DOM are present in the system and are also contributing to the total DOM pool. When the fluorescent fraction component loadings are normalized to the DOC concentration, we see that in comparison to the raw loading, they decrease over time in the stream. The DOC concentration measures both the fluorescent and non-fluorescent carbon fractions of DOM. These decreases with no concurrent increase in any of the component loadings suggest that the strong increase in DOC concentration within the stream is largely influenced by the non-fluorescent fraction of the

DOM. This trend was unexpected as major rivers show low concentrations (1-2%) of hydrolysable neutral sugars (typically a large component of the non-fluorescent fraction of the DOM) due to their rapid degradability by microbial populations (Findlay & Sinsabaugh 2003). Due to the position of this catchment at the beginning of the river continuum (Vannote et al. 1980), our results indicate that this non-fluorescent fraction of the DOM is not entirely processed out from the system by the time it reaches the stream during snowmelt. This might not be true during low flow events, where tracer experiments have shown hyporheic exchange to be rapid at both Gordon Gulch (Gabor 2013) and an alpine stream located above Gordon Gulch (Miller et al. 2006). Hyporheic exchange causes further sorption of DOM molecules, as the streamwater enters and exits the hyporheic zone. It is also possible that this non-fluorescent DOM also contains more recalcitrant materials than is typically assumed. These molecules may not be as easily degraded, such as highly aromatic molecules or molecules with fewer available exchange sites.

4.3. Spectroscopic shifts in stream source water

Spectroscopic indices from fluorescence analysis support a transition to terrestrially-derived humic materials throughout the snowmelt flushing period. This trend has been documented at other snowmelt-driven sites in Colorado (Hood et al. 2003, Hood et al. 2005, Miller & McKnight 2010). Our observed increase in the humification index (HIX) suggests a transition to more humified chemicals, with greater aromaticity and higher molecular weight (Ohno et al. 2002). High microbial activity in shallow soils (Eilers et al. 2012) rapidly degrades terrestrially-sourced DOM and increases the degree of humification of the DOM. The increase in the humification seen in the stream DOM is most likely caused by the mobilization of these shallow soil sources.

This is supported by the decrease in the freshness index (BIX), suggesting that stream DOM transitions from a more freshly-produced source (likely of microbial origin) to an older, terrestrial source (Parlanti et al. 2000). When using the freshness index, it is important to consider that this index has been primarily used in marine environments and understanding its properties in freshwater systems is an area of new research. However, the diagnostic peaks to determine the freshness index are still present in freshwater systems; therefore, we saw no reason to exclude it from analysis.

An increase in the fluorescence index (FI) towards more microbial DOM in the stream seems to disagree with the findings of an increase in terrestrial sources to the stream. However, the fluorescence index is relatively low in the stream through the entire snowmelt season, and covers a narrow range of 1.29 – 1.36 (compared to a mean of 1.58 in the groundwater). This indicates that the fluorescence index is predominately dominated by terrestrial sources (McKnight et al. 2001). This increase in the fluorescence index has a maximum of 1.36 while the average fluorescence index on the north-facing slope is 1.39. Increasing fluorescence index values in the stream further suggests that hydrologic connectivity is engaged between the shallow soils on the north-facing slope and the stream during snowmelt. The FI in the stream at the beginning of snowmelt is lower than the north-facing slope. It is possible that this indicates high microbial activity in the stream during lower flow periods, where microbes are preferentially degrading microbially-derived fulvic acids, with sp^2-hypbridized carbon atoms that are more aliphatic in nature (McKnight et al. 2001). This leaves behind the terrestrially sourced aromatic molecules which cause low FI values.

4.4. Inferences regarding catchment hydrology

Our results showing increased contribution of humic-like terrestrial material during flushing are consistent with previous studies showing an increase in terrestrial catchment sources during events due to increased hydrological connectivity of the catchment soils to the stream (Hood et al. 2003, Nguyen & Shin 2010, Inamdar et al. 2013). A closer look at the mobility of specific chemical fluorophores using PARAFAC analysis infers a transition from deep groundwater sources to the stream to soil water sources during snowmelt. We found that along the hydrograph peak in the stream, we see a concurrent rise in the groundwater table, peaking three days after discharge. This suggests that during snowmelt, vertical percolation of water to depth is rapid. However, the stream DOM chemistry does not reflect a groundwater source during snowmelt, but rather begins to look like water originating from shallow soils. This suggests that during snowmelt, infiltration is rapid in both the vertical and lateral directions, but lateral flowpaths originating from shallow soil waters are the dominant control on stream chemistry. As the shallow soils become saturated with snowmelt, the hydraulic conductivity increases, causing rapid lateral transport of soil water to the stream. These lateral flowpaths travel through the soil, where sorption processes typically remove hydrophobic molecules. In our study, the mobility of classes of fluorophores containing both hydrophilic and hydrophobic components suggests that hydrologic transport is occurring rapidly and is overwhelming DOM sorption processes onto soil surfaces. It is evident by the rise in the groundwater table that water is also flowing vertically to bedrock. However, the increase in humic-fraction in the stream reflects a soil water source, indicating that the input of lateral flow to the stream is greater than the input from groundwater.

Expression of DOC concentration in the stream has been attributed to the hydrologic connectivity of the stream to different topographic units within the catchment (Boyer et al. 1997, Boyer et al. 2000, Inamdar et al. 2004). This can create what has been referred to as 'biogeochemical hotspots and hot moments', where specific portions of the landscape have higher reaction rates in both space and time (McClain et al. 2003). Hydrologic connectivity to near-surface soils during peak discharge has shown peak DOC concentrations along the ascending limb of the stream hydrograph, due to flushing of a limited supply of DOC in shallow soils (Hornberger et al. 1994, Boyer et al. 1997), while other studies have shown peak DOC concentration on the tailing limb of the stream hydrograph when hydrologic connectivity of carbon-rich topographic units is engaged (Inamdar et al. 2004). Peak DOC concentration in our study occurs exactly with peak stream discharge, indicating that our soils are not completely flushed of DOC during snowmelt, and rather are controlled by the hydrologic forcings in the watershed. Our semi-arid catchment system lacks extensive carbon-rich topographic units such as wetlands, and has a limited riparian corridor (Hinckley et al. 2012), thus making the upland soils a more important contributor to DOC export. No temporal decrease in the DOC in hillsope soil water further suggests that these soils are not completely flushed of DOC during snowmelt. Previous studies showing DOC flushing in Colorado have been performed at higher elevation sites (Boyer et al. 1997, Hood et al. 2003). It is possible that a smaller snowpack at our lower elevation montane site results in less total infiltration to the soil during snowmelt, and does not completely flush out the DOC despite overall low C content in the soil.

5. Conclusion

We measured DOM chemistry daily across a headwater catchment in the Rocky Mountains of Colorado. We used PARAFAC component loadings to trace temporal changes in

the hydrologic connectivity of the terrestrial hillslope to the stream. We found that the hydrologic connectivity of different hillslope units is responsible for temporal changes in stream chemistry. Humic-like fluorescent DOM increases in the stream, reflecting greater hydrologic connectivity of shallow soils to the stream during snowmelt. Despite the observed increase in fluorescent humic-like DOM, we found that the non-fluorescent carbon fraction of DOM is a greater contributor to increases in total DOC concentration in the stream. Our study demonstrates how PARAFAC analysis can be used to refine our conceptual models of runoff generation processes as well as provide a more detailed understanding of stream chemistry dynamics. Further work is required to understand the spatial and temporal variability in mobile DOM throughout our study site and other montane catchments.

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